Percentiles for left ventricular rotation: comparison of reference values to paediatric patients with pacemaker-induced dyssynchrony

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Aim
Left ventricular rotation is an interesting mechanism to investigate patients with heart disease. In children, reference values have to be defined prior to assess pathology.

Methods
One hundred and seventy-four healthy individuals (0–20 years) were investigated by two-dimensional speckle tracking echocardiography, percentiles were created addressing the amount and time-to-peak values (TTP) of rotational parameters normalized to percentage of cardiac cycle (cc). Patients with right ventricular (RV) pacemaker stimulation were integrated into percentiles describing their rotational delay. Feasibility was 87.4% in healthy individuals (8.5 ± 6.2 years), 42 patients (13.0 ± 6.6 years, mean RV-stimulation time: 6.1 ± 4.3 years) were enrolled. Apical rotation (Rotap) varied and was higher than basal rotation (Rotbas) throughout all ages. Peak torsion (Tormax) normalized to left ventricle (LV) length (Tormaxi) was elevated in early childhood and decreased until adulthood. TTP values revealed greater dispersion between apical and basal rotation at younger age and a decrease during maturation. Patients with RV pacing had decreased Tormax (10.0 ± 6.0 vs. 13.7 ± 6.6°, P < 0.05), Rotap (6.7 ± 4.8 vs. 9.3 ± 5.7°, P < 0.05) and Rotbas (−3.3 ± 2.6 vs. −4.5 ± 2.7°, P < 0.05). Patients with impaired ejection fraction (EF) had abnormal delays between Rotap and Rotbas.

Conclusions
Percentile illustrations of LV rotation reveal a high amount and dispersion of Rotap and Tormax in young children as well as a higher rotational delay compared with older ages. Abnormal delays in RV pacing are associated with reduced EF.

Keywords
Torsion • Rotation • STE • Children • Percentiles • Pacemaker

Introduction
Left ventricular rotation results from interactions of a complex myocardial fibre structure. An often used construct of explanation implies the right-handed orientation of subendocardial fibres, circumferential orientation of mid-myocardial fibres, and left-handed orientation of subepicardial fibres. During systole activation of subepicardial fibres creates deformation and torque, whereas subendocardial fibres are being stretched. The resulting rotational movement starts to be reversed during the isovolumetric relaxation period. It is associated with the release of restoring forces having been accumulated in systole and is thought to create suction to facilitate diastolic filling. The nomenclature of this twisting motion is variable due to differences of methodology and modalities used for measurements. In this article, we apply the term torsion for the instantaneous angle between counterclockwise apical and clockwise basal rotation regarding the left ventricle (LV) from below. After validation against sonomicrometry and cardiac magnetic resonance quantification has become possible using echocardiography with promising results regarding accuracy.
and reproducibility.\textsuperscript{10,11} Because of applicability and the quick workflow, STE is widely used for these measurements.\textsuperscript{12–14} An interesting topic is the role of rotation in patients with dyssynchrony.\textsuperscript{15–17} Children with congenital and acquired heart disease have not been investigated sufficiently concerning the chronology of rotation if they are dependent on pacemakers. It was the aim of our study to generate percentiles for rotational parameters from 0 to 20 years with a special interest on chronology. Thereafter, we investigated pacemaker patients with chronic atrial ventricular (RV)-pacing in order to detect the effects of pacemaker-induced dyssynchrony on left ventricular rotation.

Methods

Study description

The project was performed between March 2009 and December 2011. Written informed consent from parents, legal guardians or caretakers was obtained for echocardiographic investigations that were approved by the institutional ethics committee (Reg.-Nr.05/2009). One hundred and seventy-four healthy children and adolescents in sinus rhythm could be included to obtain reference values after cardiovascular disease was ruled out by the standard echocardiography. Fifty-five patients with pacemakers, who were examined on a routine control basis in our outpatients’ clinic were enrolled to assess the clinical applicability of the reference values.

Ultrasound

A Vivid 7\textsuperscript{®} ultrasound machine (GE Medical Systems, Milwaukee, WI, USA) was used equipped with 7S, 5S, and M3S probes (range 3–7 MHz) with frame-rates ranging from 55 to 90 frames/s and second harmonic imaging. All individuals were examined in a supine left lateral position. For STE, scanning of left ventricular short-axis loops at apical and basal levels was performed. Specific attention was paid on a cross section as circular as possible. At the mitral valve level, care was taken not to include atrial tissue. To obtain a proper view of the apical rotation, exclusion of papillary muscles and the lowest slice with persisting lumen was mandatory. The optional transducer position was one to two intercostal spaces below the standard short-axis views. End-expiratory breath-hold was performed only if image quality was limited and compliance was sufficient. Aortic and mitral valve Doppler signals were taken to establish timing events in combination with ECG signals. All examinations were performed by the same investigator.

Data analysis was done on a personal computer workstation with the Echopac-Software (Version 6.1.2, GE Medical Systems) by an independent investigator in a blinded fashion.

Angular displacement of the LV determined by use of the two-dimensional-strain software at the apical and mitral valve level is referred to as rotation expressed in degrees (deg) in this article. Briefly, the software calculates the mean rotational values from six myocardial segments within each slice that were used to calculate Rotap, Rotbas, and peak systolic torsion (Tor\textsubscript{max}). This was defined as the maximum instantaneous net-difference of values between the apex and basis of the LV and was secondarily normalized to left ventricular length, i.e. normalized peak systolic torsion (Tor\textsubscript{max}) (deg/cm) = (apical rotation – basal rotation)/left ventricular diastolic longitudinal length. Chronology was assessed by defining the time-to-peak value (TTP) rotational value, normalized to the percentage of cardiac cycle (cc, Figure 1). To define the delay between Rotap and Rotbas, TTP values were subtracted.

In pacemaker patients, left ventricular ejection fraction (EF) and dysynchrony indices (SDI 17) were assessed using a V3 matric probe, RT3DE data sets were analysed using the LV-Analysis 2.7 software (Tomtec, Unterschleissheim, Germany).

Statistics

Statistical analysis was performed using software package SPSS, version 21 (Chicago, IL, USA). Pearson’s correlation, Student’s t-test, the ANOVA-test, and the Kruskal–Wallis and Mann–Whitney test were used. Reference centile curves were computed with the LMS method by Cole (Lambda for the skew, Mu for the median and Sigma for the coefficient of variation), using the LMS version 1.27 software (Institute of Child Health, London, UK).\textsuperscript{18}

Intra- and inter-observer variability had been assessed in a previous study\textsuperscript{19} and was not re-evaluated because of good comparability to other studies. To assure sufficient number of individuals for every age group, sample size calculation was carried out in 50 individuals with $\alpha = 0.05$ and $\beta = 0.90$ with a difference of 10% to be detected.\textsuperscript{20}

Results

Demographic data

A total of 152 healthy children and adolescents from 0 to 20 years ($8.5 \pm 6.2$, median 9.5) could be used for entire quantification of data (87.4%). Some data sets had to be excluded because of limited image quality or technical problems. Sample size calculation resulted in six individuals necessary to detect a 10% change in Tor\textsubscript{max}. Data were, therefore, subdivided into five minor age groups (0–1, 2–6, 7–10, 11–14, 15–20 years, Table 1 and 2) for further statistical workup.

Rotation

Apical rotation

Rotap was always counterclockwise in all age groups with two peaks in infancy and at the age of 10 to 12 years (Figure 2A). In all, it provides a highly variable range from 2 to 30°. In adulthood Rotap shows a smaller bandwidth and a decrease compared with infancy and toddler’s age. No correlations were found between the amount of Rotap, heart rate (HR), body surface area (BSA), height, weight, age, and sex. Significant differences were found between group 1 and 2, group 1 and 5, group 2 and 4, and group 4 and 5. TTP values of Rotap normalized to the percentage of cc shorten during maturation (Figure 2B). There was a negative correlation with HR ($r = -0.69, P < 0.05$), positive correlations with height ($r = 0.57, P < 0.05$), weight ($r = 0.56, P < 0.05$) and age ($r = 0.58, P < 0.05$), correlation with BSA was weak ($r < 0.5, P < 0.05$). Significant differences between group 1 and 5 could not be detected investigating all possible combinations.

Basal rotation

Rotbas occurs in opposite to apical rotation. Therefore, values generated are negative values. Data are only presented as absolute values in the percentiles for reasons of calculation. There was exclusively clockwise rotation remaining relatively constant during all ages with a range from 0 to –11 (Figure 3A). There were no correlations between the amount of Rotbas, HR, BSA, height, weight, age, and sex. No differences between group 1 and 5 could be detected investigating all possible combinations.
TTP values of Rotbas normalized to cc shorten with increasing age to a higher extent than do Rotap TTP values (Figure 3B). Significant correlations were found with HR ($r = 0.64$, $P < 0.05$), height ($r = 0.48$, $P < 0.05$), weight ($r = 0.48$, $P < 0.05$), age ($r = 0.51$, $P < 0.05$), only weak correlation with BSA. Significant differences were detected between group 1 and group 3, group 1 and group 4, and group 1 and group 5.

Peak systolic torsion
$T_{\text{ormax}}$ has a peak in infancy and at the age of 10–12 years, similar to $\text{Rot}_{\text{ap}}$, with a bandwidth from 3 up to 34°, stabilizing in early adulthood (Figure 4A). There are no correlations with HR, BSA, height, weight, age, and sex. Comparison of the subgroups did not result in additional differences.

There is a correlation between TTP $T_{\text{ormax}}$ normalized to cc and HR ($r = -0.7$, $P < 0.05$), height ($r = 0.57$, $P < 0.05$), weight ($r = 0.56$, $P < 0.05$), and age ($r = -0.6$, $P < 0.05$). Regarding the percentage of cc, $T_{\text{ormax}}$ is completed at 60% in infancy and decreases to 50% in adulthood (Figure 4B).

Normalized peak systolic torsion
Normalization to left ventricular length shows a high range of $T_{\text{ormax}}$ in infancy, stabilizing in adulthood (Figure 5). Statistical significance was only evident between group 3 and 5 ($P < 0.05$) and 4 and 5.

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**Figure 1:** Rotation curves graphic illustration of clockwise (negative values) and counterclockwise (positive values) global apical (turquoise), basal (purple) rotation, and torsion (white) in degree. Rot$_{\text{ap}}$, Rot$_{\text{bas}}$, and Tor$_{\text{max}}$ are the maximum values of the curves, TTP values are measured from the beginning of the R-wave to maximum (see arrows).

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**Table 1** Demographic data of healthy individuals

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BSA (m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>0–20 (8.5 ± 6.2, median 9.5)</td>
<td>77 f, 75 m</td>
<td>122.9 ± 46.5 (42–196)</td>
<td>33.2 ± 24.4 (1.6–100)</td>
<td>1.04 ± 0.6 (0.14–2.28)</td>
</tr>
<tr>
<td>35</td>
<td>Group 1 0–1 (0.06 ± 0.16, median 0.05)</td>
<td>15 f, 20 m</td>
<td>50.6 ± 6.9 (42–74)</td>
<td>3.4 ± 1.7 (1.6–9.8)</td>
<td>0.21 ± 0.06 (0.14–0.43)</td>
</tr>
<tr>
<td>21</td>
<td>Group 2 2–6 (3.8 ± 1.6, median 3.9)</td>
<td>12 f, 9 m</td>
<td>100.3 ± 17.2 (52–120)</td>
<td>15.5 ± 5.0 (3.2–23)</td>
<td>0.65 ± 0.17 (0.21–0.87)</td>
</tr>
<tr>
<td>25</td>
<td>Group 3 7–10 (8.2 ± 1.2, median 8.3)</td>
<td>14 f, 11 m</td>
<td>132.7 ± 9.9 (110–145)</td>
<td>29.4 ± 5.7 (20–39.3)</td>
<td>1.0 ± 0.14 (0.8–1.3)</td>
</tr>
<tr>
<td>42</td>
<td>Group 4 11–14 (12.3 ± 0.9, median 12.4)</td>
<td>22 f, 20 m</td>
<td>154.8 ± 10 (125–172)</td>
<td>45.4 ± 10.6 (31–69)</td>
<td>1.4 ± 0.19 (1.1–1.81)</td>
</tr>
<tr>
<td>29</td>
<td>Group 5 15–20 (16.7 ± 1.9, median 16.4)</td>
<td>14 f, 15 m</td>
<td>172.1 ± 11.1 (151–196)</td>
<td>67.6 ± 13.6 (46.8–100)</td>
<td>1.79 ± 0.22 (1.43–2.28)</td>
</tr>
</tbody>
</table>

Data as mean ± standard deviation, range in rounded brackets.
Echocardiographic rotational parameters of healthy individuals

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Rate (bpm)</th>
<th>Rot ap (°)</th>
<th>TTP (sec)</th>
<th>Tor max (%cc)</th>
<th>Delay (%cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>127 ± 18 (85–168)</td>
<td>14.6 ± 8.6 (0.8–32)</td>
<td>60.6 ± 4.5 (0.3–32)</td>
<td>16.1 ± 12.1 (38–89)</td>
<td>12.7 ± 5.4 (10–38)</td>
</tr>
<tr>
<td>Group 2</td>
<td>125 ± 11 (58–107)</td>
<td>13.2 ± 8.6 (3–30.8)</td>
<td>55.2 ± 3.1 (6.3–83)</td>
<td>15.7 ± 16.1 (7.9–74)</td>
<td>12.2 ± 5.4 (10–59)</td>
</tr>
<tr>
<td>Group 3</td>
<td>88 ± 11 (53–107)</td>
<td>12.0 ± 8.3 (3–32)</td>
<td>54.5 ± 2.7 (1.2–30.3)</td>
<td>11.4 ± 10.1 (26.7–70.1)</td>
<td>11.4 ± 6.1 (10–59)</td>
</tr>
<tr>
<td>Group 4</td>
<td>71 ± 14 (39–103)</td>
<td>9.8 ± 4.3 (1.7–34.4)</td>
<td>54.6 ± 4.1 (2.9–38)</td>
<td>12.7 ± 6.2 (35.7–83.4)</td>
<td>9.8 ± 5.4 (35.7–83.4)</td>
</tr>
<tr>
<td>Group 5</td>
<td>98 ± 15 (79–230)</td>
<td>6.9 ± 2.6 (1.9–38.9)</td>
<td>54.5 ± 4.3 (2.9–38)</td>
<td>12.7 ± 5.4 (35.7–83.4)</td>
<td>9.8 ± 5.4 (35.7–83.4)</td>
</tr>
</tbody>
</table>

Comparison of pacemaker patients with healthy controls

A healthy control group was compared with each group. A pacemaker group showed higher peak QRS, longer QRS duration, and a higher RR interval compared with the healthy control group. There were no significant differences in age, height, weight, and sex among the groups.

Table 2: Echocardiographic rotational parameters of healthy individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (sec)</td>
<td>0.67 ± 0.07</td>
<td>0.6 ± 0.05</td>
<td>0.54 ± 0.06</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>QRS (sec)</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>QTc (sec)</td>
<td>0.39 ± 0.03</td>
<td>0.38 ± 0.03</td>
<td>0.38 ± 0.03</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>TTP (sec)</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

Comparison of pacemaker patients with healthy controls

A healthy control group was compared with each group. A pacemaker group showed higher peak QRS, longer QRS duration, and a higher RR interval compared with the healthy control group. There were no significant differences in age, height, weight, and sex among the groups.
indices (SDI 17) revealed a negative correlation with Tor max ($r = -0.42$, $P < 0.05$) and EF ($r = -0.66$, $P < 0.05$). A transfer of all delays between apical and basal rotation into the created percentiles of all controls shows that patients with reduced EF have a delay outside a double standard deviation ($>90\%c$ or $<10\%c$), whereas delays of patients with normal EF were situated more closely to normal delays (Figure 6).

**Discussion**

This study provides reference percentiles for left ventricular rotational parameters covering infants, children, and young adults. In addition, clinical usefulness was investigated in a group of paediatric patients with chronic RV-pacing with potential dyssynchrony. Major findings of the study are (i) that Tor max is higher during infancy than proven in former studies with lower sample size, (ii) that TTP rotational values shorten during ageing if they are

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**Table 3** Demographic data of patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Sex (female, male)</td>
<td>22, 20</td>
<td>22, 20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13.0 ± 6.6</td>
<td>12.2 ± 5.5a</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>146.5 ± 28.2</td>
<td>149.3 ± 32.2a</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.8 ± 23.5</td>
<td>47.9 ± 25a</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.36 ± 0.46</td>
<td>1.40 ± 0.52a</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>57 ± 20</td>
<td>65 ± 16a</td>
</tr>
<tr>
<td>Rotap (°)</td>
<td>6.7 ± 4.8</td>
<td>9.3 ± 5.7b</td>
</tr>
<tr>
<td>TTP Rotap (%cc)</td>
<td>48.6 ± 17</td>
<td>45.5 ± 10a</td>
</tr>
<tr>
<td>Rotbas (°)</td>
<td>$(-3.3) ± 2.6$</td>
<td>$(-4.5) ± 2.7b$</td>
</tr>
<tr>
<td>TTP Rotbas (%cc)</td>
<td>52.8 ± 21</td>
<td>60 ± 13.5a</td>
</tr>
<tr>
<td>Tor max (°)</td>
<td>10.0 ± 6.0</td>
<td>13.7 ± 6.6b</td>
</tr>
<tr>
<td>Delay between Rotmax and Rotap (°/cc)</td>
<td>4.2 ± 27.6</td>
<td>14.5 ± 14b</td>
</tr>
</tbody>
</table>

Data as mean ± standard deviation.

*a*No statistical significance.

*b*Significance with $P < 0.05.$
of HR. Our observation showed slightly higher TTP values in infancy and toddlers’ age which shorten at older age groups; this finding is consistent with the transition of HR controlled into volume controlled cardiac workout where diastole is expanding in percentage of cc during maturation. Thus, the approach of normalizing the TTP values to the percentage of cc might be advantageous for clinical use. An early systolic basal counterclockwise rotation was evident throughout all ages, whereas TTP could be related to the end of systole and TTP values of clockwise Rotbas were related to early diastole. Even in our groups of young adults the finding of identical TTP values of Rotap and Rotbas was not achieved. This had been reported in studies about the importance of TTP Rotap and TTP Rotbas for the early diastolic untwisting, mitral valve opening and early diastolic E-wave flow. Under stress the delay between Rotap and Rotbas becomes longer leading to an enhanced apex-to-base gradient of relaxation to facilitate diastolic filling, but this is due to shortening of TTP Rotap. TTP Rotbas observed in infants is so late that it either hints at ineffective untwisting or a higher electromechanical delay. Nevertheless, effective cardiac output in younger children seems to be less dependent on the timing of Rotbas than in older children where elastic properties and increased volume loading under stress become more important.

Magnitude of rotation

The first surprising result of our investigation is the presence of slightly higher absolute and as considerably higher normalized Tormax during infancy and toddler’s age when compared with early adulthood. This was predominantly evoked by an enhanced Rotap. These results are partly in agreement with the first important study investigating maturational changes by Notomi et al. who demonstrated a slightly higher Tormax in infancy using tissue Doppler in a total of 25 children of different age as well as in a very recent study using 3D speckle-tracking Takahashi et al. and Al-Naami did not report about changes in normalized Tormax during maturation using a comparable methodology. Our study included more than double the number of children and had a higher sample size than the other studies. The methodology by using percentiles is potentially more suitable when investigating maturational changes in parameters with high standard deviations that were also reflected in the studies cited. Interestingly, standard deviations decreased until adulthood reflecting the importance of torsion as an individual and situational variable component of LV work enhancement in young hearts not being able to change their filling volumes substantially.

Timing of rotation

The second important observation is that TTP of Rotap, Rotbas, and Tormax decreases if values are normalized to percentage of cc. This is important as HR highly correlates with timing of rotational values and might lead to confusing results especially in children who can present with a high variability of HRs. Normalization to the cc results in an increased delay between Rotap and Rotbas at younger age. This was also observed in the study of van Dalen who could show a decreasing delay between Rotap and Rotbas in young adults. Al-Naami reported about an increase in TTP values for Tormax but in this study there was no compensation for the influence of HR. Our observation showed slightly higher TTP values in infancy and toddlers’ age which shorten at older age groups; this finding is consistent with the transition of HR controlled into volume controlled cardiac workout where diastole is expanding in percentage of cc during maturation. Thus, the approach of normalizing the TTP values to the percentage of cc might be advantageous for clinical use. An early systolic basal counterclockwise rotation was evident throughout all ages, whereas TTP could be related to the end of systole and TTP values of clockwise Rotbas were related to early diastole. Even in our groups of young adults the finding of identical TTP values of Rotap and Rotbas was not achieved. This had been reported in studies about the importance of TTP Rotap and TTP Rotbas for the early diastolic untwisting, mitral valve opening and early diastolic E-wave flow. Under stress the delay between Rotap and Rotbas becomes longer leading to an enhanced apex-to-base gradient of relaxation to facilitate diastolic filling, but this is due to shortening of TTP Rotap. TTP Rotbas observed in infants is so late that it either hints at ineffective untwisting or a higher electromechanical delay. Nevertheless, effective cardiac output in younger children seems to be less dependent on the timing of Rotbas than in older children where elastic properties and increased volume loading under stress become more important.

Pacemaker-induced dyssynchrony

A significant deviation from a normal delay in the timing of rotation can be found in patients with chronic RV pacemaker stimulation and impaired cardiac function had abnormal delays between Rotap and Rotbas. This could either mean too close or too wide, in 15 patients it was even reversed. Thus, there might be an ‘optimal’ time-span between first apical and then basal rotation which is important for appropriate cardiac function. The results are undermined by positive correlations between EF and Tormax on the one hand and negative correlations between dyssynchrony indices, Tormax and EF on the other hand. The topic of twist mechanics in the assessment of cardiac dyssynchrony is being intensively investigated at the moment. A reduction of magnitude in Tormax in dyssynchrony has been reported in studies on adult patients. Especially in cardiac resynchronization therapy heart failure with normal EF and dilated cardiomyopathy the role of rotation in dyssynchrony seems to play an important role for diagnostic and prognostic reasons. The therapeutic relevance for CRT indication remains to be investigated further, the reference values presented here might be helpful in this process.

Limitations

Our study has some limitations that have to be discussed. First, we only investigated systolic events. The literature states the importance of untwisting for diastolic function. Having in mind the limited temporal resolution of STE in combination with high HRs in young children we refrained from including these measurements into our study.

Second exclusive measurement of Tormax using basal and apical two-dimensional slices has limitations that could in part be overcome using 3D STE. Unfortunately, RT3DE is much more demanding for recording the entire apex has lower temporal resolution and quantification of rotation is currently not available by all vendors.
Finally, additional echocardiographic data about healthy children and pacemaker patients would have given even more information about correlations between rotational and well-established dysynchrony parameters. We refrained from that because of the amount of data to be included.

**Conclusion**

We have created percentiles for the amount and timing of left ventricular rotation in a large cohort of individuals from 0 to 20 years. Maturational changes and pacemaker-induced dysynchrony serving as a useful field of application are presented and may demonstrate the importance of reference values also for the use in other pathologies.

**Conflict of interest:** none declared.

**References**